

Distribution of the second virial coefficients of globular proteins

Richard P. Sear

Department of Physics, University of Surrey, Guildford, Surrey GU2 7XH, United Kingdom

r.sear@surrey.ac.uk

February 1, 2008

Abstract

George and Wilson [Acta. Cryst. D 50, 361 (1994)] looked at the distribution of values of the second virial coefficient of globular proteins, under the conditions at which they crystallise. They found the values to lie within a fairly narrow range. We have defined a simple model of a generic globular protein. We then generate a set of proteins by picking values for the parameters of the model from a probability distribution. At fixed solubility, this set of proteins is found to have values of the second virial coefficient that fall within a fairly narrow range. The shape of the probability distribution of the second virial coefficient is Gaussian because the second virial coefficient is a sum of contributions from different patches on the protein surface.

PACS: 87.14.Ee, 87.15.Nn.

Protein crystallisation is an important problem yet our grasp of the details of how it occurs is very poor. Proteins need to be crystallised from solution in order to determine their structure via X-ray crystallography [1, 2]. The crystallisation presumably starts with heterogeneous nucleation of the crystalline phase in the protein solution, but there has been no systematic experimental study of this, as far as the author is aware. Without an understanding of how proteins crystallise, protein crystallisation is almost totally *ad hoc*: essentially the only way to know if a protein will crystallise under a certain set of conditions is to try it. It would be enormously useful if we could *predict* the conditions under which a protein was most likely to crystallise. Here by protein we mean globular protein, which are proteins that are soluble in solution, as opposed to membrane proteins which exist embedded in a surfactant bilayer. The hope that it is possible to predict the conditions that promote crystallisation motivated George and Wilson [3] to look at the values of the (osmotic) second virial coefficient of a number of proteins under the conditions where they were crystallised. They found that the second virial coefficient was always negative and lay within what they called ‘a fairly narrow range’. If we ignore outliers then second virial coefficients gathered together by Haas and Drenth [4], and converted to reduced units by Vliegthart and Lekkerkerker [5], lie in the range -8 to -40 , in units of the volume of the protein; see Table V of Ref. [5].

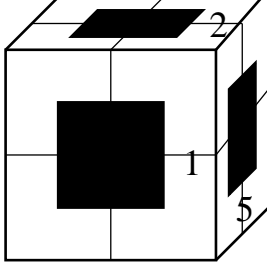
The simplest explanation of this range is that the upper limit is set by the requirement that the attractive interac-

tions be strong enough to pull the molecules into a crystal from a dilute solution. The lower limit is set by the dynamics of the solution, if the attractive interactions are too strong the protein molecules tend to aggregate irreversibly and this aggregation preempts and prevents crystallisation. Testing these explanations is all but impossible due to our poor understanding of crystallisation so we turn to a well-defined, and easily calculated, property of a protein solution: its solubility. We consider the solubility of the protein, i.e., the concentration of protein in the solution which coexists with the crystal, in preference to the process of crystallisation. We ask the question: For a given solubility, say 5% by volume, what is the distribution of values that we expect for B_2 ? If we have 1000 proteins, say, all with the same solubility, then is their distribution of values of B_2 very broad, or is it narrow? What is the shape of the distribution, i.e., what is its functional form?

The distribution of values of B_2 of a large number of proteins defines a probability distribution function $P(B_2)$. We will consider a constraint, that of fixed solubility, and so will obtain a probability distribution function that also depends on this constraint. We are inspired to study this function by a range of work on protein solutions and crystals [6–12] that has shown that protein-protein interactions are well described by a sum over contacts between the proteins. Where by a contact between two proteins, we mean that a specific patch on the surface of one protein approaches closely, a couple of Å, to a specific patch on the surface of the other protein. Now, if these contacts are more-or-less independent then we expect them to contribute essentially independently to B_2 . But if B_2 is the sum of many independent contributions for each protein, then we know the form of the distribution function $P(B_2)$: it is a Gaussian. The central limit theorem states that the probability distribution function of some property Y , which is a sum over a large number of independent random variables, is a Gaussian [13]. Also of course the more independent patches there are on the surface the narrower will be the distribution of values of B_2 .

The physical picture is that the surface of a protein has a number of patches on its surface. Under the conditions where the protein’s solubility is low, these patches attract each other. The strength of each patch attraction is then a random variable selected from some distribution. It is a random variable if the strength of the attraction of one patch on the surface is independent of the attraction of

Figure 1: A schematic of our model protein. It is drawn as a cube with the attractive patches drawn as black patches on the faces of the cube. The model occupies 2 by 2 by 2 = 8 lattice sites. The individual lattice sites occupied by the model are separated by the thinner lines.



any of the other patches. This physical picture is very simple and is of course approximate but if the correlations between the various patches are weak then $P(B_2)$ should be approximately Gaussian. Of course this will also apply to the probability distribution function of any other variable which is a sum of more-or-less independent contributions from the surface patches. We do not know whether the rate of crystallisation is such a variable.

Before we consider our model it is worthwhile noting that within biology there is a move away from studying proteins one at a time to studying them en masse, e.g., studying the complete proteome of an organism. Where the proteome is defined as being the complete set of proteins possessed by an organism. This follows on from work on establishing the complete genome of a number of organisms [14, 15]. Although here we consider sets of proteins which are just those we want to crystallise, and so can come from a number of different organisms, in the future the solubilities and virial coefficients of complete proteomes could be considered.

The model is chosen to be as simple as possible, while incorporating the patchy nature of the surface of proteins together with the variability in the interactions from protein to protein. Thus for simplicity we chose a lattice model. The lattice is cubic and each protein occupies 8 lattice sites arranged 2 by 2 by 2, see Fig. 1. We make the model 2 sites across to reduce the range of the attraction, which is 1 site, to half the diameter of the hard core. The model ‘proteins’ can rotate, and so have 24 distinct orientations. Each of the 6 faces of the cube has a patch, labeled $i = 1$ to 6, with patches 1 to 4 clockwise around a loop of 4 of the faces, and patches 5 and 6 on the remaining 2 faces. The interactions between model proteins are pairwise additive and consist of 2 parts. The first is simply an excluded volume interaction: 2 proteins cannot overlap. The second is that if the faces of 2 proteins are in contact there is an energy of interaction between the 2 touching patches of the 2 proteins. By in contact we mean that *all* of the 4 lattice sites on the face of one protein are in contact with one of the lattice sites of the face of the other protein, in other words the faces must overlap completely otherwise the energy of

interaction is taken to be zero. The touching patches are those on the faces of the 2 proteins that face each other. If the i th patch of one protein is adjacent to the j th patch of another protein then there is an interaction energy of ϵ_{ij} . Different proteins will have the same excluded volume interactions but the set of interactions ϵ_{ij} will be different to represent the different surfaces of different proteins. The interactions form a symmetric square matrix, $\epsilon_{ij} = \epsilon_{ji}$.

Thus, a protein is specified by giving values to the 21 distinct ϵ_{ij} ; these are composed of 36 interactions, consisting of 6 like interactions, $i = j$, and 30 unlike interactions, $i \neq j$, with 15 of these related to the other 15 by symmetry. The energies ϵ_{ij} determine the phase behaviour via Boltzmann weights $b_{ij} = \exp(-\epsilon_{ij}/kT)$, where k is Boltzmann’s constant and T is the temperature. We will in fact deal mainly with these weights not with the energies themselves.

For the values of the b_{ij} for the patch-patch interactions we again choose the simplest possible model and neglect any correlations. Each of the 21 distinct b_{ij} is *almost* a stochastic variable described by a probability function $p(b)$, i.e., the probability that b_{ij} lies between b and $b+db$ equals $p(b)db$. They are almost but not quite uncorrelated because we want the crystalline phase of all our model proteins to be a simple cubic lattice with 1 protein molecule per unit cell. The crystal is close-packed; there is a protein on every lattice site. In such a crystal, 3 different bonds are formed: the 13, 24 and 56 bonds and the energy e_x is

$$\begin{aligned} e_x &= \epsilon_{13} + \epsilon_{24} + \epsilon_{56} \\ \frac{e_x}{kT} &= -\ln(b_{13}b_{24}b_{56}) \end{aligned} \quad (1)$$

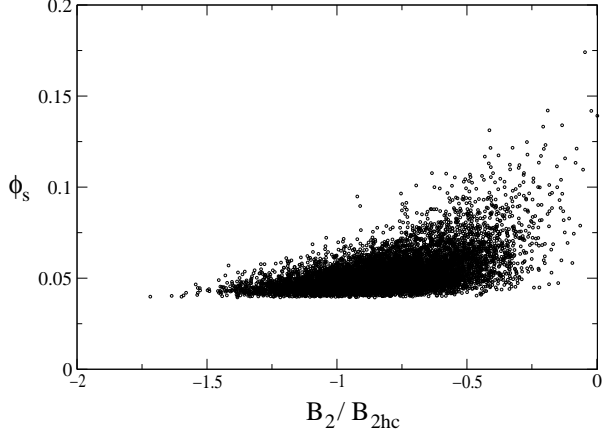
We want this to be the ground state and so having generated the 21 distinct Boltzmann weights, we find the 3 largest of these, call them $b_{\alpha\beta}$, $b_{\gamma\delta}$ and $b_{\delta\zeta}$ and then we interchange b_{13} and $b_{\alpha\beta}$, b_{24} and $b_{\gamma\delta}$ and b_{56} and $b_{\delta\zeta}$. In this way we ensure the simple cubic lattice is the ground state of the model protein. Performing this swapping procedure introduces correlations of course, but they are minor as we have ordered only 3 of the 21 weights.

The entropy of the crystalline phase is zero, as the cubes can neither translate nor rotate, so its free energy is equal to its energy, e_x . At low temperature, the pressure will be low and so the chemical potential will be closely equal to the free energy, which we have already said is equal to the energy. Thus the chemical potential in the crystalline phase $\mu_x \approx e_x$. The chemical potential in a dilute solution, which is well described by an ideal gas, is

$$\mu_{ig} = kT \ln(\rho/24), \quad (2)$$

where the 24 comes from the rotational entropy $k \ln 24$ of a freely rotating cube with all 6 faces distinct. ρ is the number density, the number of proteins per lattice site. When a dilute solution coexists with the crystal then we can treat the solution as an ideal gas and find the density of this solution by equating μ_{ig} of Eq. (2) with μ_x , which is closely equal to e_x of Eq. (1). So, the density of the solution that coexists with the crystal, i.e., the solubility of

Figure 2: A scatter plot of the the solubility volume fraction ϕ_s , the y axis, against the reduced second virial coefficient B_2/B_{2hc} , the x axis, for 10,000 proteins. The mean weight $\bar{b} = 9$ and the distribution is a top-hat with width $b_w = 16$.



the protein is

$$\rho_s = 24 \exp(e_x/kT). \quad (3)$$

A more useful measure of density is the volume fraction $\phi = 8\rho$ which is the fraction of the sites in the fluid occupied by the proteins. Thus we will work in terms of the solubility volume fraction $\phi_s = 8\rho_s$.

The second virial coefficient of a continuum model is defined by

$$B_2 = -\frac{1}{2} \int d\mathbf{r}_{12} d\omega_1 d\omega_2 [\exp(-u(\mathbf{r}_{12}, \omega_1, \omega_2)) - 1], \quad (4)$$

where \mathbf{r}_{12} is the distance between the centres of mass of two molecules, and ω_1 and ω_2 are the orientations of molecules 1 and 2, respectively. The integrations over the angles are normalised. $u(\mathbf{r}_{12}, \omega_1, \omega_2)$ is the energy of interaction of pair of molecules, as a function of their separation and orientation. For a lattice model the integrals are replaced by sums and we have

$$B_2 = -\frac{1}{1152} \sum_{\alpha=1}^{24} \sum_{\beta=1}^{24} \sum_{\{\mathbf{r}_{12}\}} [\exp(-u(\mathbf{r}_{12}, \alpha, \beta)) - 1], \quad (5)$$

where the three sums are, in order, over the 24 orientations of molecule 1, over the 24 orientations of molecule 2 and over the separation of the two molecules. The factor of 1152 comes from the factor of half and two factors of 24 from the normalisation of the sums over orientation.

The second virial coefficient B_2 of the protein-protein interaction can be calculated once the set of weights b_{ij} are specified. The exponential factor in Eq. 5 equals 0 if the two molecules overlap and equals one of the b_{ij} when the

molecules occupy adjacent sites. Thus we have

$$B_2 = \frac{1}{2} \left[27 - \frac{1}{6} \sum_{i=1}^6 \sum_{j=1}^6 (b_{ij} - 1) \right], \quad (6)$$

where the first term inside the brackets comes from excluded volume interactions and the second from the interactions between touching patches. The number 27 comes from the fact that each model protein excludes other proteins from a cube of 3 by 3 by 3 lattice sites. Thus, in the high temperature limit where $b_{ij} = 1 \forall i, j$, $B_2 = B_{2hc} = 27/2$. The sums over 24 orientations reduce to sums over 6 orientations as rotating either of the 2 molecules around the axis joining their centres does not change the energy. The factor in front of the double sum is a normalisation factor of $1/36$ times the 6 possible lattice sites that one molecule can occupy and be adjacent to the other molecule.

Finally, for the purposes of performing example calculations we will take the distribution function $p(b)$ to be a top-hat function of mean \bar{b} and width b_w .

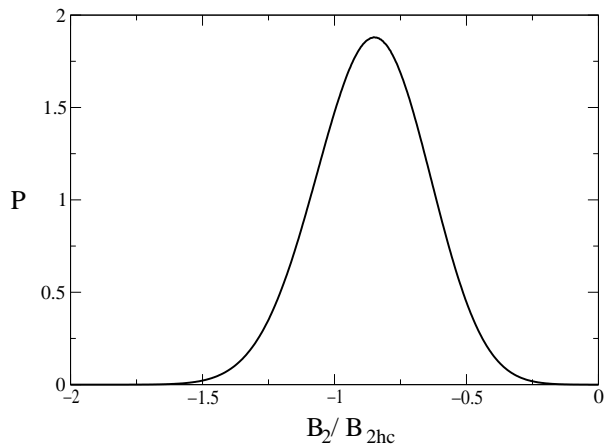
$$p(b) = \begin{cases} 0 & b < \bar{b} - b_w/2 \\ b_w^{-1} & \bar{b} - b_w/2 \leq b < \bar{b} + b_w/2 \\ 0 & b > \bar{b} + b_w/2 \end{cases} \quad (7)$$

With our model defined we can generate a protein by generating values for the 21 distinct Boltzmann weights for the patch-patch interactions, b_{ij} , according to the probability distribution function $p(b)$. Repeating this procedure many times will generate a set of proteins, each protein having a distinct array of interactions, b_{ij} . This set can then be analysed to look, for example, for correlations between different properties.

Before we look at a set of proteins with solubilities in a fixed, small, range let us look at just a set of proteins, with a range of solubilities. Fixing the mean patch-patch interaction weight $\bar{b} = 9$ and the width $b_w = 16$ so that the Boltzmann weights lie in the range 1 to 17, we have generated a set of 10,000 proteins. Humans have about 10 times as many different proteins as this, bacteria typically less than half this number [15]. The mean weight was chosen to give a distribution centred around $\phi_s \approx 0.05$. Of course increasing \bar{b} will shift the distribution to lower values of ϕ_s and decreasing \bar{b} will shift the distribution to higher values of ϕ_s . Their solubilities and second virial coefficients are plotted as a scatter plot in Fig. 2. Clearly, there is a correlation between the second virial coefficient of a protein and its solubility: the more negative is B_2 the lower is the solubility on average, but there is also considerable scatter.

Now, restricting the solubility of a protein to lie in the narrow range $\phi_s = 0.05 \pm 0.01$, we have calculated the probability distribution of second virial coefficients and plotted the result in Fig. 3. The curve is almost Gaussian; a Gaussian is a good fit to the data. The standard deviation is close to 0.2. We have used a top-hat probability distribution function for the Boltzmann weights of patch-patch interactions, Eq. 7, but of course our finding of a Gaussian distribution is insensitive to the exact form of this distribution function.

Figure 3: A plot of the probability distribution function $P(B_2/B_{2hc})$ of the reduced second virial coefficient B_2/B_{2hc} , for model proteins with a solubility in the range $\phi_s = 0.05 \pm 0.01$. As in Fig. 2, $\bar{b} = 9$ and $b_w = 16$.



There is relatively little data for proteins where both the second virial coefficient and the solubility have been measured. Extensive results are available for lysozyme [16, 17], but there are results for both the virial coefficient and the solubility for only a couple of other proteins. Rosenbaum *et al.* [17] plot the reduced second virial coefficient against solubility of lysozyme under a number of different conditions, γ -crystallin and BPTI (bovine pancreatic trypsin inhibitor), their Fig. 4. With only 3 proteins no attempt can be made at assessing a probability distribution. However, in the wake of the sequencing of whole genomes there is a drive towards so-called ‘high throughput’ methods which can rapidly assess the properties of large numbers of proteins. If such a method could be developed for second virial coefficients the results could be compared to our predictions. For a given protein there is clear experimental evidence that as conditions are varied so as to make the second virial coefficient more negative the solubility decreases [12, 18]. For our model, this corresponds to increasing the mean Boltzmann weight of the attractions, \bar{b} , which will decrease both B_2 and the solubility, whatever the distribution of weights for the patch-patch attractions.

We have swapped 3 of the patch-patch interaction energies to force the ground state to be a simple cubic lattice. Because of this, when the attractions are made strong enough, \bar{b} sufficiently positive, our model proteins all have low solubilities. Thus they are presumably representative of proteins that are easily crystallisable. If we had not swapped the 3 interaction energies, and we assume that the only possible crystalline phase is the simple cubic, then some of our model proteins will be highly soluble: they will not crystallise from dilute solutions.

In conclusion, the surfaces of proteins are patchy and mediate short-range interactions, short with respect to the size of the protein, which is a few nms. A quantity such

as the second virial coefficient is an integral (sum for lattice models) over the contribution of the core of the protein, over the contributions of any attractions between patches of the surface, and over the contribution of any longer-ranged interactions such as an overall electrostatic repulsion. If the patches are independent or almost independent, then the distribution function for their total contribution to the second virial coefficient, of a large number of proteins of a similar size will tend to be Gaussian. A straightforward consequence of the central limit theorem. The central limit theorem applies to a variable, here the second virial coefficient, that is the sum of a large number of random variables with the same mean and variance. The suggestion is that if experiments are done on a large set of proteins all at the same solubility and all with similar sizes, then this fixed solubility will set a rough scale for the mean strength of the interactions. Then if proteins are highly modular, the predominant variation from protein to protein will come from variations in the sum of the patch-patch Boltzmann weights, and so will have a roughly Gaussian distribution. With a width roughly equal to the square root of the number of independent patches times the width in the distribution of the Boltzmann weight of a single patch. Thus, a second virial coefficient which is a sum over contributions from a number of independent patches will tend to have a narrow distribution of values. This may be partly responsible for the fairly narrow range of values of the second virial coefficient over which most proteins crystallise [3–5] but crystallisation is both complex and poorly understood so there are almost certainly other factors. By highly modular we mean that the patches on a protein’s surface are close to being independent. If they were truly independent we would have $\langle b_{ij}b_{i'j'} \rangle = \bar{b}^2$, $ij \neq i'j'$, where $\langle \rangle$ indicates an average over all the proteins, and the interactions ij and $i'j'$ are any two patch-patch interactions of the same protein. We are assuming that violations of this equality are weak.

It is a pleasure to acknowledge discussions with J. Cuesta, D. Frenkel and P. Warren.

References

- [1] DURBIN S. D. and FEHER G., *Ann. Rev. Phys. Chem.*, **47** (1996), 171.
- [2] PIAZZA R., *Curr. Opinion Coll. Int. Sci.*, **5** (2000), 38.
- [3] GEORGE A. and WILSON W. W., *Acta. Cryst. D*, **50** (1994), 361.
- [4] HAAS C. and DRENTH J., *J. Phys. Chem. B*, **102** (1998), 4426.
- [5] VLIEGENTHART G. A. and LEKKERKERKER H. N. W., *J. Chem. Phys.*, **112** (200), 5364.
- [6] NEAL B. L., ASTHAGIRI D., VELEV O. D., LENHOFF A. M. and KALER E. W., *J. Cryst. Growth*, **196** (1999), 377.

- [7] HAAS C., DRENTH J. and WILSON W. W., *J. Phys. Chem. B*, **103** (1999), 2808.
- [8] SEAR R. P., *J. Chem. Phys.*, **111** (1999), 4800.
- [9] CARLSSON F., MALMSTEN M. and LINSE P., *J. Phys. Chem. B*, **105** (2001), 12189.
- [10] CURTIS R. A., BLANCH H. W. and PRAUSNITZ J. M., *J. Phys. Chem. B*, **105** (2001), 2445.
- [11] WARREN P. B., *J. Phys.: Cond. Matt.*, in press; cond-mat/0201418.
- [12] KULKARNI A. and ZUKOSKI C. F., preprint.
- [13] MA S.-K., *Statistical Mechanics* (World Scientific Press, Singapore, 1985).
- [14] INTERNATIONAL HUMAN GENOME SEQUENCE CONSORTIUM, *Nature*, **409** (2001), 860; VENTER J. C., *et al.*, *Science*, **291** (2001), 1304. These 2 papers are drafts of the human genome, for their relevance to the proteome, see FIELDS S., *Science*, **291** (2001), 1221.
- [15] The amino acid sequences of the complete set of proteins of a number of organisms are available at <http://www.ebi.ac.uk/proteome/>.
- [16] GUO B., KAO S., MCDONALD H., ASANOV A., COMBS L. L. and WILSON W. W., *J. Cryst. Growth*, **196** (1999), 424.
- [17] ROSENBAUM D. F., KULKARNI A., RAMAKRISHNAN S. and ZUKOSKI C. F., *J. Chem. Phys.*, **111** (1999), 9882.
- [18] POON W. C. K., EGELHAAF S. U., BEALES P. A., SALONEN A. and SAWYER L., *J. Phys. Cond. Matt.*, **12** (2000), L569.